PB83-213926

Characterization of the DNA of a Nonoccluded Baculovirus, Hz-1V

(U.S.) Health Effects Research Lab.
Research Triangle Park, NC

1982

U.S. Department of Commerce
National Technical Information Service
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TECHNICAL REPORT DATA
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1. REPORT NO. EPA-600/J-82-113	2.
3. R PB83-213926	
4. TITLE AND SUBTITLE Characterization of the DNA of a Nonoccluded Baculovirus, Hz-1V (Journal Version)	5. REPORT DATE 1982
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) Yuan-Shen Huang, M. Hedberg and C.Y. Kawanishi	8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS Health Effects Research Laboratory US Environmental Protection Agency Research Triangle Park, NC 27711	10. PROGRAM ELEMENT NO.
	11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS Office of Research & Development Health Effects Research Laboratory US Environmental Protection Agency Research Triangle Park, NC 27711	13. TYPE OF REPORT AND PERIOD COVERED
	14. SPONSORING AGENCY CODE EPA-600/11

15. SUPPLEMENTARY NOTES

Published In: Journal of Virology, 1982, 174-181

16. ABSTRACT

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17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT Release to Public	19. SECURITY CLASS (This Report) Unclassified	21. NO. OF PAGES 9
	20. SECURITY CLASS (This page) Unclassified	22. PRICE

EPA Form 2220-1 (Rev. 4-77) PREVI

REPRODUCED BY NATIONAL TECHNICAL INFORMATION SERVICE U.S. DEPARTMENT OF COMMERCE SPRINGFIELD, VA. 22161

EPA-600/J-82-113
JOURNAL ARTICLE

JOURNAL OF VIROLOGY, July 1982, p. 174–181
0022-538X/82/070174-08$02.00/0

Vol. 43, No. 1

Characterization of the DNA of a Nonoccluded Baculovirus, Hz-1V

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Received 28 December 1981/Accepted 29 March 1982

The DNA of the nonoccluded baculovirus (Hz-1V) obtained from the IMC-Hz-1 cell line was characterized by physicochemical and restriction endonuclease techniques. Hz-1V DNA isolated from purified virus had buoyant densities of 1.58 and 1.54 g/ml in CsCl-ethidium bromide density gradients, which corresponded to supercoiled and to relaxed circular and linear DNA, respectively. Neutral CsCl equilibrium centrifugation indicated that the Hz-1V DNA had a buoyant density of 1.7024 g/ml, which corresponded to a guanine-plus-cytosine (G+C) content of 43%. Thermal denaturation indicated a high G+C domain(s) in the Hz-1V genomic DNA. The domain(s), which included about 11% of the total genomic DNA, exhibited a T_m of 97°C. The remaining portion (89%) of the DNA had a T_m of 86.5°C. The T_ms corresponded to G+C contents of 42 and 67%, respectively. The mean genetic complexity of Hz-1V DNA determined by DNA reassociation kinetic analysis was found to be 152×10^6. A possible rapidly reassociating component comprising approximately 13% of the genome was observed. The mean molecular weights from restriction endonuclease digests were 159×10^6 for both *Hin*dIII and *Eco*RI. Genomic heterogeneity was found in both the wild-type Hz-1V stock and in two plaque isolates. Of 12 single-plaque isolates, 3 basic restriction endonuclease DNA fragment patterns were observed. The molecular size estimates from electron microscopic contour lengths of uncloned viral DNA ranged from 70 to 158 megadaltons, and the mode was the 130- to 140-megadalton class.

A baculovirus agent that persistently infects the IMC-Hz-1 cell line from several sources was first reported by Granados et al. (3). The presence of this and other adventitious agents in cell cultures that are being developed for propagation of viruses as active ingredients for pesticides are of safety concern (3, 7, 15). The possibility that Hz-1V was a cell culture-adapted strain of the *Heliothis zea S* (singly enveloped) nuclear polyhedrosis virus (NPV), which is the active component of a registered biorational pesticide, its propensity to establish persistent infections (18a), and its inducibility from such cultures by other baculoviruses (9) enhance its potential as a contaminant. For these reasons, the agent was further characterized. We report here the details of the properties of the Hz-1V DNA. A less detailed study was published earlier by Langridge (12).

MATERIALS AND METHODS

Cells and virus. TN-368 cell culture methods, Hz-1V source and production, and plaque assay methods were described previously (18a). Briefly, TN-368 cells, grown in TNM-FH medium of Hink (4), were infected with Hz-1V at a multiplicity of 0.01 and incubated at 28°C. Extracellular virus was harvested from culture media 48 h after infection. Hz-1V plaque isolates were obtained by three successive cycles of single-plaque isolation and propagation.

Virion purification. The harvested culture medium was pooled and centrifuged at $2,000 \times g$ for 10 min to remove cells and cellular debris. The supernatant was layered on 5 ml of 30% sucrose in 50 mM Tris-hydrochloride, pH 7.6–1 mM EDTA in a Beckman SW27 nitrocellulose tube followed by centrifugation at $100,000 \times g$ for 30 min. The virus pellets were resuspended in standard citric saline (SSC), which contains 0.15 M NaCl–0.015 M sodium citrate (pH 7.2) buffer, and were centrifuged for 60 min as before. The milky virus band between 50 and 55% sucrose was collected, diluted with an equal volume of SSC, and recovered by centrifugation at $100,000 \times g$ for 30 min. The virus pellets were resuspended in SSC and stored at −20°C until used.

Isolation of virus DNA. Solutions of virus at a concentration of 1 mg/ml in SSC were mixed with 1% sodium Sarkosyl and 0.5 mg of protease K. The solution was incubated at 37°C for 4 h and was layered on a continuous CsCl-ethidium bromide (200 μg/ml) gradient (1.36 to 1.7 g/ml). The centrifugation was carried out in an SW41 rotor at $150,000 \times g$ for 16 to 18 h at 20°C. The gradient was fractionated through the bottom of the tube, and the densities of the fraction

were determined with a Bausch & Lomb ABBE-34 refractometer. The two DNA bands visualized by UV light were pooled. Ethidium bromide was removed by repeated extraction of the DNA solution with isoamyl alcohol. The solution was then dialyzed overnight with 0.1× SSC and was stored at −20°C.

Isopycnic centrifugation. The density of Hz-1V DNA was determined by equilibrium CsCl density gradient centrifugation. The DNA in 0.1× SSC was slowly mixed with solid CsCl to give a solution with a final density of 1.700 g/ml and centrifuged at 150,000 × *g* at 20°C for 72 h in a Beckman T65 fixed-angle rotor. Lambda phage DNA with a density of 1.709 was used as a marker. When [^{32}P]DNA was used, the gradient was fractionated, and the Cerenkov count was determined for each fraction with a liquid scintillation counter. The guanine-plus-cytosine (G+C) content of Hz-1V DNA in mole fraction was calculated according to Schildkraut et al. (21). Ethidium bromide-CsCl gradient centrifugation and identification of form I and II DNA were carried out as described by Radloff et al. (18) and Summers and Anderson (26).

Thermal denaturation and reassociation kinetics. For thermal denaturation and reassociation kinetics studies, Hz-1V DNA was prepared as follows: 40 μg of Hz-1V DNA in 1 ml of 0.12 M sodium phosphate buffer, pH 6.8, was sheared by sonication with a Branson sonic oscillator to an average size of 0.5×10^6 daltons as determined by agarose gel electrophoresis. The sheared DNA was dialyzed against three changes of 0.12 M sodium phosphate buffer, pH 6.8, over a 24-h period.

Thermal denaturation was carried out in a Gilford model 250 spectrophotometer with attached model 2527 thermal programmer. Thermal denaturation was accomplished by heating from 60 to 100°C at a programmed rate of 1°C/min, and absorbance was recorded at 15-s intervals. Lambda phage DNA with a G+C content of 49% was used as a reference. The G+C content of Hz-1V DNA was calculated from the T_m according to Marmur and Doty (14).

Reassociation kinetic analysis was carried out with the heat-denatured DNA by monitoring the rate of hypochromic change at 260 nm as a function of time after the temperature shift to 60°C. DNA concentration was determined from the initial absorbance at 260 nm with lambda phage DNA as a standard. $C_0t_{1/2}$ curves were determined by the renaturation experiments according to Britten and Kohne (1) and Wetmur and Davidson (28) to estimate the genomic complexity of Hz-1V DNA. Differences arising from variance in G+C content between Hz-1V DNA and the lambda standard were corrected according to Seidler and Mandel (22).

Restriction enzyme analysis and autoradiography. One microgram of ^{32}P-labeled Hz-1V was incubated at 37°C with 5 U of *Eco*RI or *Hin*dIII restriction endonuclease (Bethesda Research Laboratories) for 4 h in 20 μl of solution containing 10 mM Tris-hydrochloride, pH 7.5, 10 mM $MgCl_2$, 6 mM β-mercapthoethanol, and 0.1 M NaCl. Reactions were stopped by the addition of 2 μl of 0.1 M EDTA containing 0.05% bromophenol blue–50% glycerol followed by heating at 60°C for 5 min. The reaction mixtures were loaded onto a vertical 0.8% agarose slab gel for electrophoresis in a buffer containing 40 mM Tris, pH 7.8, 20 mM sodium acetate, and 1 mM EDTA. Electrophoresis was performed at 2 V/cm until the dye marker reached the bottom of the gel. The gel was dried under vacuum on filter paper. Autoradiograms were obtained by exposing Kodak X-Omat-R film to the dried gel for 24 to 48 h. The molecular weight of each Hz-1V DNA fragment was determined by its mobility relative to *Eco*RI or *Hin*dIII fragments of λ DNA (27).

Electron microscopy. Contour length and configuration of Hz-1V DNA were examined by the technique of Kleinschmidt et al. (10) with minor modifications. Ten microliters of Hz-1V DNA (≅1 μg/ml) was added to a 100-μl solution containing 10 μl of ammonium acetate (5.0 M, pH 7.5), 12 μl of cytochrome *c* (100 μg/ml), and 5 μl of PM-2 phage DNA (1 μg/ml) as an internal size marker (17). The pH of ammonium acetate was checked before each spreading. The DNA solution was rolled down a glass slide at a 20 to 30° incline onto a 0.25 M ammonium acetate (pH 7.5) hypophase. The DNA solution was dispensed slowly from a syringe with a 16-gauge needle with an attached piece of wide-bore tubing to prevent shearing.

The DNA-protein films were picked up from the hypophase on Parlodion-coated copper grids (200 mesh) at different time intervals (2 to 10 min). The grids were stained for 30 s in nearly saturated uranyl acetate in 75% ethanol, rinsed for 30 s in 75% ethanol, and air dried. The grids were rotary shadowed with platinum-palladium (80:20) at an angle of approximately 7°. Micrographs were taken only of circular DNA with a Philips EM300 electron microscope. The measurements of the DNA molecules were made with a Hewlett-Packard digitizer.

RESULTS

Yield and isopycnic centrifugation of viral DNA. High-speed pellets of culture medium from 2×10^8 TN-368 cells infected with Hz-1V yielded about 100 to 150 μg of viral DNA. By centrifugation in a neutral CsCl gradient, buoyant density was determined to be 1.7024 g/ml for Hz-1V DNA (Fig. 1). The G+C content of Hz-1V DNA was estimated to be 43 mol%.

Equilibrium centrifugation of Hz-1V DNA in ethidium bromide-CsCl density gradients resulted in two bands with densities of 1.58 and 1.54 g/ml, corresponding to supercoiled and relaxed circular and linear forms, respectively, seen with other baculovirus DNA.

Thermal denaturation. The thermal denaturation curve for the Hz-1V DNA was biphasic. A T_m of 86°C was obtained for the major fraction of Hz-1V DNA in 0.12 M sodium phosphate buffer, pH 6.8, using *Autographa californica M* NPV (Ac*M*NPV) and phage lambda DNA as a reference. In addition, a minor domain(s) of Hz-1V genomic DNA denatured between 94 to 99.6°C (Fig. 2) and represented approximately 11% of the total genome. The G+C contents calculated according to Marmur and Doty (14) were 43 and 68% for the major and minor DNA domains of Hz-1V, respectively.

Reassociation kinetics. The genomic complexity of Hz-1V DNA was determined by reassocia-

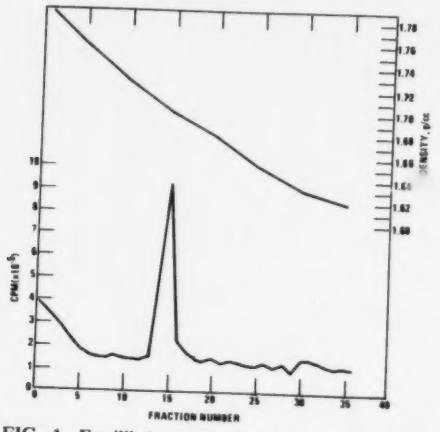

FIG. 1. Equilibrium centrifugation of Hz-1V DNA in neutral cesium chloride. ^{32}P-labeled Hz-1V DNA was centrifuged at 150,000 × *g* at 20°C for 72 h. The Cerenkov radiation and refractive index were determined for the fractions. A buoyant density of 1.7024 g/ml and 43 mol% G+C content were established for Hz-1V DNA.

tion kinetic analysis, using phage lambda DNA as a standard (Fig. 3). Under these experimental conditions, the $C_0t_{1/2}$ value determined for lambda DNA was 0.076, and that for Hz-1V DNA was 0.41 mol·s/liter. Relative to the molecular weight of 30.8×10^6 for lambda DNA (6, 27), the $C_0t_{1/2}$ value of Hz-1V DNA corresponded to a genomic size of approximately 166×10^6 daltons.

Correction for the difference in G+C content (22) between the two DNAs gave a refined genomic size estimate of 154×10^6 daltons. A second determination (not shown) gave a complexity estimate of 150×10^6 daltons. The mean of the two values was 152×10^6 daltons.

The course of reassociation of Hz-1V DNA suggested a biphasic curve. If it was indeed biphasic, the two slopes of the reassociation curve indicated that approximately 13% of the Hz-1V DNA genome comprised repeated sequences.

Electron microscopy of Hz-1V DNA. Electron microscopic examination revealed freshly isolated unfrozen Hz-1V DNA in supercoiled, partially relaxed or relaxed circular (Fig. 4) molecules. Table 1 presents the observed contour lengths and calculated size estimates of the circular molecules, relative to circular PM-2 DNA internal standard of molecular weight 3.7×10^6 (17). The data show heterogeneity of Hz-1V DNA contour lengths with molecular weights ranging from 75×10^6 to 158×10^6. The mean molecular weight and standard deviation were $123 \pm 20 \times 10^6$. The modal class was in the range of 131 to 140.

Restriction endonuclease studies. Results of agarose gel electrophoresis resulting after digestion of the DNAs of 12 Hz-1V plaque isolates with *Eco*RI and *Hin*dIII restriction endonucleases were analyzed. Figure 5 shows the three basic restriction patterns evident with both enzymes. The most common pattern (7 of 12 isolates) was that shown by isolate 179. Three other isolates exhibited the pattern represented by isolate 170. The remaining two are exemplified by the pattern of 181. Isolate 179 was most similar to the pattern of the unplaqued original virus stock.

Isolate 170 (Fig. 6) was designated as the standard Hz-1V DNA for molecular weight determination. Submolar bands were not observed in this isolate. It also had the smallest molecular weight with both enzymes used.

Digestion of Hz-1V isolate 170 DNA with *Hin*dIII resulted in 28 fragments ranging in size from 0.65×10^6 to 11.8×10^6 daltons (Table 2). Twenty-one were obtained with *Eco*RI of sizes 0.4×10^6 to 19.2×10^6 daltons (Table 3). *Hin*dIII bands A and K and *Eco*RI band J were quantitatively 2 M. Submolar bands were not observed. The cumulative molecular weight of *Hin*dIII fragments of isolate 170 DNA was 157×10^6 and was 155×10^6 for *Eco*RI.

Submolar bands were observed in the *Hin*dIII

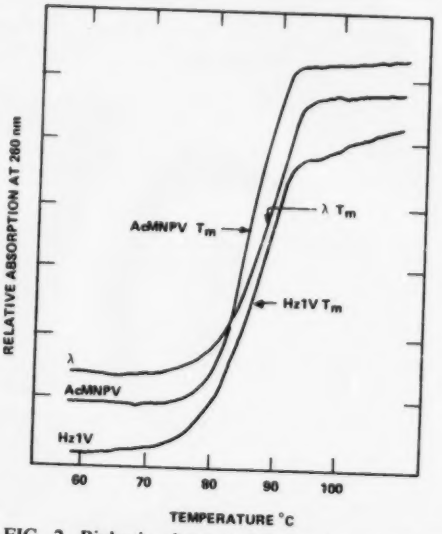

FIG. 2. Biphasic thermal denaturation curve of sheared Hz-1V DNA in 0.12 M sodium phosphate buffer, pH 6.8. Ac*M*NPV and phage lambda DNAs were included as references. The T_m points are indicated by arrows. A second, more slowly denaturing fraction is evident above 94°C for the Hz-1V curve compared with those of lambda and Ac*M*NPV. This indicates that mir or domains of high G+C content occur in the Hz-1V DNA.

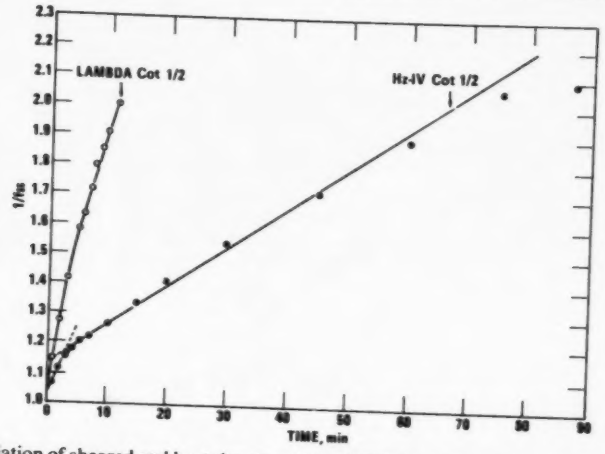

FIG. 3. Reassociation of sheared and heat-denatured Hz-1V DNA at 60°C in 0.12 M sodium phosphate buffer, pH 6.8. Hz-1V genomic DNA appears to exhibit a minor rapidly renaturing fraction indicative of reiterated sequences and a more slowly reassociating phase. Relative to phage lambda DNA, a mean genomic complexity corrected for difference in G+C content of 154×10^6 can be calculated for Hz-1V DNA from the ratios of $C_0t_{1/2}$ values.

fragment patterns of isolate 181 and 179 DNAs. Both DNAs were larger than isolate 170 DNA. The electrophoretic mobility of the *Hin*dIII L band (4.9×10^6) of isolate 181 decreased to a position corresponding to a molecular weight of 5.17×10^6. The K band (5.4×10^6) of isolate 179 was shifted to a position with a molecular weight of 5.85×10^6. Stoichiometrically, the molar ratios of bands B, C, F, G, H, T, and U were less than 1 relative to other bands in isolate 181. New submolar bands N′ and S′ were found in isolate 179 with apparent molecular weights of 3.85×10^6 and 2.7×10^6, respectively. The total molecular weight of isolate 181 was 157×10^6, and that of isolate 179 was 163×10^6, with equal consideration of the submolar bands. The DNA restriction pattern of the original wild-type virus showed little difference from 179 except for the absence of the N′ band.

*Eco*RI digestion of isolate 181 DNA showed a new band, L″, with a corresponding molecular weight of 3.95×10^6. This band was not found in isolate 170 DNA. Bands B, E, D, and F also showed submolarity in 181. The cumulative molecular weight of isolate 181 was 159×10^6. Viral DNA of isolate 179 exhibited new bands at L′,

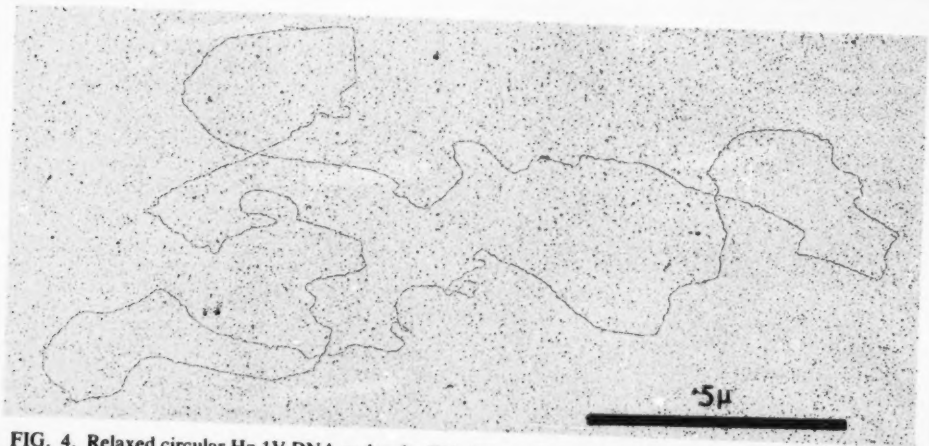

FIG. 4. Relaxed circular Hz-1V DNA molecule. This is one of several configurations in which the genome of this virus, including supercoiled and linear, can be isolated. Hz-1V DNA resembles those of other baculoviruses in this characteristic.

TABLE 1. Molecular weights of Hz-1V DNA molecules determined by electron microscopy

Mol wt (10^6)	No. of molecules[a]
71–80	2
81–90	4
91–100	1
101–110	1
111–120	8
121–130	8
131–140	10
141–150	7
151–160	1

[a] Total = 42; range = 75×10^6 to 158×10^6; mean = $123 \pm 20 \times 10^6$.

P′, R′, and S′ positions with corresponding molecular weights of 4.12, 2.0, 1.24, and 0.68×10^6. Shifting of band I resulted in a molecular weight change from 7.3×10^6 to 7.48×10^6. The total molecular weight of isolate 179 DNA was calculated as 163×10^6 based on such analysis. The *Eco*RI digestion pattern of wild-type viral DNA was most similar to that of isolate 179.

DISCUSSION

The DNA of Hz-1V was observed to be supercoiled, circular double-stranded DNA. This may be true for DNAs of all baculoviruses (25). Centrifugation in CsCl-ethidium bromide density gradients resolved the Hz-1V DNA into zones corresponding to supercoiled covalently closed DNA and to relaxed circular and linear forms of DNA at the expected densities of 1.58 and 1.54 g/ml, respectively. These two components showed no difference in the DNA fragment patterns after digestion with restriction endonucleases (Y.-S. Huang, M. Hedberg, and C. Y. Kawanishi, unpublished data). The presence of these forms was confirmed by electron microscopy.

The buoyant density of Hz-1V DNA was determined to be 1.7024 g/ml in neutral CsCl density gradient compared with lambda DNA (density, 1.705 g/ml) standard and TN-368 cellular DNA (1.702 g/ml). The G+C content of Hz-1V DNA calculated on the basis of buoyant density equilibrium centrifugation in neutral CsCl gradients was about 43 mol%. This estimate is in good agreement with 42 mol% derived from the T_m. These values are nominal for baculoviruses (25) and coincide with that of the nonoccluded baculovirus of *Oryctes rhinoceros* (16). The thermal denaturation curve (Fig. 2) of purified extracellular Hz-1V DNA was shown to be biphasic. It indicated a minor domain(s) with high G+C content in the Hz-1V genomic DNA.

The DNA buoyant densities of baculoviruses reported vary over a wide range, from 1.694 g/ml in the case of *Adoxophyes orana* NPV (5) to

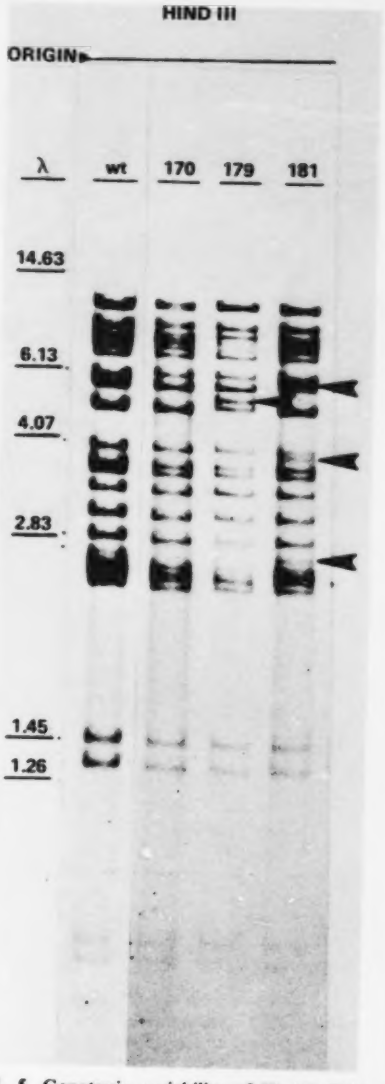

FIG. 5. Genotypic variability of Hz-1V DNA revealed by restriction endonuclease digestion. The DNAs of the original unplaque-purified virus (wild type) and plaque-purified isolates 170, 179, and 181 are shown. These isolates represent the 3 basic autoradiographic fragment patterns obtained from 12 isolates after agarose gel (0.8%) electrophoresis of restriction endonuclease-digested [^{32}P]DNA. Fragments unique to each of the three basic patterns are indicated by arrowheads. The position of coelectrophoresed phage lambda DNA fragment markers and their sizes in megadaltons are presented as references.

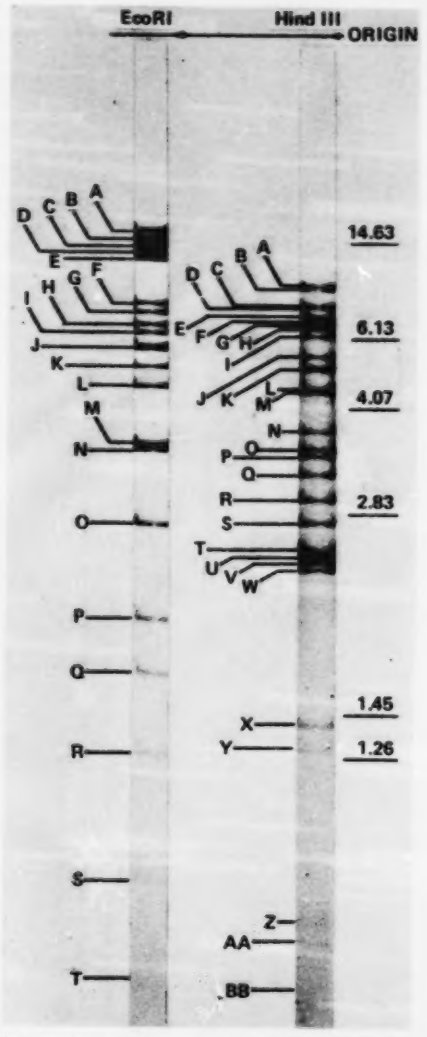

FIG. 6. Autoradiogram of restriction endonuclease *Eco*RI and *Hin*dIII fragments of Hz-1V isolate 170 DNA. Electrophoretic separation of the [^{32}P]DNA fragments was carried in a vertical 0.8% agarose slab gel. *Hin*dIII digest of phage lambda DNA was coelectrophoresed as reference, and the positions and sizes of the marker fragments are indicated. Isolate 170, one of the most homogeneous of the isolates, showed cumulative molecular weights of 155 × 10^6 by *Eco*RI and 157 × 10^6 by *Hin*dIII.

1.715 g/ml for *Orgyia pseudotsugate M*NPV (19). In this study, Hz-1V DNA had a slightly higher density than those of *H.zea S*NPV, *Spodoptera frugiperda M*NPV, and Ac*M*NPV.

The three independent estimates of the molecular weight of Hz-1V DNA in this study indicated that it is the largest among the known circular supercoiled viral DNAs. Analyses of *Hin*dIII and *Eco*RI fragments of three isolates indicate molecular weight estimates of between 155 × 10^6 and 163 × 10^6, with a mean of 159 × 10^6. These data approximate the genetic complexity estimate of 152 × 10^6 obtained by kinetic analysis of DNA reassociation. By electron microscopy, however, the contour lengths of Hz-1V DNA molecules varied greatly, from 76 × 10^6 to 158 × 10^6. The modal class was found to be 130 × 10^6 to 140 × 10^6, with a mean value of 128 × 10^6. This estimate agrees more closely with the earlier estimates of Langridge (12). However, the values obtained from contour length measurements in the present study are likely to be underestimates for the following reasons. First, the DNA for contour length measurements was obtained and measured before our recognition of the presence of defective particles and the critical influence multiplicity of infection exerts over the production of such Hz-1V particles during propagation (C. Y. Kawanishi, Y.-S. Huang, and M. Hedberg, manuscript in preparation). Second, these values represent a limited sample

TABLE 2. Molecular weight determination of Hz-1 baculovirus isolate 170 DNA *Hin*dIII fragments in 0.8% agarose gel

Electrophoresis band	Mol wt (10^6)	Molar ratio	Sum[a]
A	11.8	2	23.6
B	11.2	1	11.2
C	9.8	1	9.8
D	9.5	1	9.5
E	8.5	1	8.5
F	8.3	1	8.3
G	8.1	1	8.1
H	7.8	1	7.8
I	7.3	1	7.3
J	6.3	1	6.3
K	5.4	2	10.8
L	4.9	1	4.9
M	4.75	1	4.75
N	3.95	1	3.95
O	3.7	1	3.7
P	3.65	1	3.65
Q	3.4	1	3.4
R	3.1	1	3.1
S	2.85	1	2.85
T	2.6	1	2.6
U	2.52	1	2.52
V	2.49	1	2.49
W	2.43	1	2.43
X	1.45	1	1.45
Y	1.35	1	1.35
Z	0.8	1	0.8
A′	0.75	1	0.75
B′	0.65	1	0.65

[a] Total = 156.56.

TABLE 3. *Eco*RI Fragments of Hz-1 baculovirus isolate 170 DNA in 0.8% agarose gel

Electrophoresis band	Mol wt (10^6)	Molar ratio	Sum[a]
A	19.9	1	19.9
B	17.7	1	17.7
C	16.0	1	16.0
D	14.5	1	14.5
E	13.7	1	13.7
F	9.4	1	9.4
G	8.4	1	8.4
H	7.5	1	7.5
I	7.3	1	7.3
J	6.0	2	12.0
K	5.2	1	5.2
L	4.6	1	4.6
M	3.85	1	3.85
N	3.6	1	3.6
O	2.75	1	2.75
P	2.22	1	2.22
Q	1.67	1	1.67
R	1.30	1	1.30
S	0.88	1	0.88
T	0.64	1	0.64
U	0.4	1	0.4

[a] Total = 154.81.

of individual Hz-1V DNA molecules (n = 42), whereas molecular weight estimates obtained by restriction endonuclease and reassociation kinetic analyses are averages of whole populations of molecules. Third, because the larger molecules are more likely to be sheared during preparation for viewing with an electron microscope, the size distribution may have been skewed toward the smaller classes because only intact, relaxed circles were photographed and measured.

The grand mean value of 156 × 10^6 daltons obtained in the present study from restriction endonuclease and reassociation kinetic analyses agrees with the largest DNA molecule size of 158 × 10^6 daltons observed in the electron microscope study. Such a genome size is nearly double that observed for the nonoccluded *Oryctes rhinoceros* baculovirus (16). The reason for the variance between the restriction endonuclease-derived molecular weight estimates of the present study and those of Langridge (12) remains unresolved.

Reassociation kinetic analyses also suggested the presence of repeated sequences in Hz-1V DNA. This was indicated by the presence of a rapidly reassociating component (Fig. 3), which comprised about 13% of the total Hz-1V DNA sequence. The reassociation rate of this component was two to three times faster than those of unique components. Whether the suggested repeated DNA sequences are identical to the high G+C region which accounts for 11% of the total hyperchromicity remains to be resolved. Repeated sequences have also been observed in other NPV DNAs (2, 8), but they accounted for a much smaller fraction of the total genome. No repeated sequences were detected in *A. orana*, *Barathra brassicae*, *Orgyia pseudotsugata*, and *H. zea* NPVs (5, 19, 20).

The presence of heterogeneity and genetic variability of DNA in uncloned virus preparation is documented for other baculoviruses (11, 13, 24). Thus, its demonstration for Hz-1V DNA was not unexpected. Three basic genotypes among 12 plaque isolates were revealed. Isolate 170 proved to be the most homogeneous, had no submolar restriction fragments after *Hin*dIII and *Eco*RI digestion, and had the lowest molecular weight estimate. Consequently, isolate 170 was designated as the standard for Hz-1V DNA molecular weight estimation in this study. However, DNA of Hz-1V isolate 179 was most similar to that of wild-type viral DNA. The contribution to the genomic heterogeneity of physically defective Hz-1V particles and DNA mentioned earlier, which was observed with isolates 179 and 181, specifically as they relate to the presence of submolar DNA fragments even after three plaque purifications, remains to be assessed.

Hz-1V appears unique in several characteristics. The DNA is an unusually large, covalently closed, supercoiled molecule and appears to contain high G+C domains and possibly reiterated sequences. Biologically, Hz-1V has a predisposition to establish persistent infections (3, 9, 18a). Whether these features are related is currently unknown. Our ultimate concern remains the health significance of this agent should it occur adventitiously in insect virus preparations used as pesticides. To this end, the data presented in this study contribute to the definitive identification and detection of Hz-1V.

ACKNOWLEDGMENTS

We are grateful to Ann Ralston for her assistance in the propagation of the virus. We also thank J. Griffith, of the University of North Carolina, Cancer Research Center, for advice in the preparation of DNA for electron microscopic study and for making available instrumentation for DNA length measurements.

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